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Short Communication

Effects of Dimethyl Sulfoxide and pH on Indoleacetic Acid-Induced Lateral Root Formation in the Radish Seedling Root[1]

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ABSTRACT

Segments (2.5 cm) cut from 3-day-old seedling roots of radish (*Raphanus sativus* L. 'Scarlet Globe') were cultured in medium with or without indoleacetic acid (IAA). Lateral root primordia frequency, determined for the central centimeter of segments, was dependent on IAA concentration and on conditions affecting IAA uptake. Dimethyl sulfoxide treatment, or a relatively low medium pH, greatly enhanced the response to exogenous IAA. It was concluded that a permeation barrier exists between the external medium and the hormone responsive sites within the radish seedling root.

A characteristic action of IAA is to stimulate the formation of LR.[3] This IAA effect has been studied using simple experimental systems employing excised or intact seedling roots (1, 10–13, and references cited therein). Using our system (1) based on the radish (*Raphanus sativus* L. 'Scarlet Globe') seedling root, we find that the response to exogenous auxin can be substantially modulated by chemical or physical factors known or presumed to influence diffusional IAA uptake by plant cells. The observations reported here indicate that there is an IAA permeation barrier between the external medium and the auxin-responsive sites involved with LR formation within the radish seedling root.

MATERIALS AND METHODS

These studies were performed with roots of seedlings germinated and grown in the dark at 24°C for 3 d, during which time the roots grew to an average length of 6 cm. Excised 2.5 cm segments, cut starting 0.5 to 1.5 cm behind the seedling root tip, were cultured 4 d in medium containing Murashige-Skoog salts (7), 88 mol m^{-3} sucrose, and 10 mol m^{-3} each of the buffers Mes and succinic acid. IAA and/or DMSO were added as indicated. Segments were inoculated into 25 ml aliquots of liquid medium contained in 125 ml flasks. Three to five flasks, each containing four segments, were used per treatment. Flask cultures were agitated on a rotary shaker (100 rpm) in the dark at 24°C. Sterile technique was observed throughout. After harvest and fixation, counts were made of the number of LRP occurring in the central 1 cm of each segment. Further details may be found in Blakely *et al.* (1).

RESULTS AND DISCUSSION

Exogenous IAA greatly increased the frequency (*i.e.* the number/cm) of LRP formation, which varied from 5 cm^{-1} in the absence of IAA to about 60 cm^{-1} under optimal conditions. Excess IAA inhibited LRP formation.

Figure 1 illustrates the effect of 30 mmol m^{-3} IAA, and the modulation of the response by pH and DMSO. Typical treatment mean frequencies of LRP formation in medium containing IAA were: 49.7 ± 3.6 SE LRP cm^{-1} at pH 4.5 and 19.0 ± 1.9 SE LRP cm^{-1} at pH 6. Treatment with DMSO increased the response to IAA at pH 6; a typical treatment mean frequency was 48.1 ± 3.8 SE LRP cm^{-1}.

DMSO is known to increase plasmalemma permeability to small molecules (3). In our experience, it was found most effective when given as a pulse treatment at a medium concentration of 5% (v/v) during the first 1 to 4 h of culture. Longer exposure to DMSO, or higher concentrations of DMSO, were inhibitory. DMSO by itself (*i.e.* with no exogenous auxin) had no promotive effects on the frequency of LRP formation. Administration of a 5% DMSO 4 h pulse treatment when medium containing 30 mmol m^{-3} IAA was buffered at pH 4.5, rather than 6, inhibited LRP formation.

The effect of pH on IAA uptake by plant cells has been well characterized (4, 9). Since the membrane permeability coefficient to the lipophilic IAAH is on the order of 10^3 times that of IAA^- (9), lower pH values, which inhibit dissociation, foster greater diffusional uptake. It can be calculated (9) that, at pH 4.5, 61.3% of the IAA would be in the IAAH form; at pH 6, only 4.8% would be in the IAAH form.

Further evidence of a permeation barrier was seen in the effects of injury on the response to exogenous IAA. The LRP frequency was higher at both cut ends of the 2.5 cm segments when segments were cultured in medium containing IAA, but not when cultured in medium lacking IAA. The effect extended inward 1 to 3 mm from the ends. When segments were injured with a scalpel or tweezers, the LRP frequency was higher in injured regions, but only when the medium contained IAA.

Based on these observations, it is evident that the auxin-responsive sites involved with LRP formation in the radish seedling root reside behind a permeation barrier. The pH and DMSO effects suggest that the barrier may consist, at least in part, of membranes. It is well known that the endodermis restricts apoplastic entry to the root stele in several species (8), and there is some evidence for this in the specific case of the radish primary root (6). The effect of injury may be to allow apoplastic entrance of IAA into the stele, bypassing a barrier at the endodermis. The permeation barrier may therefore consist of (a) an apoplastic

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[3] Abbreviations: LR, lateral root(s); LRP, lateral root primordia(um); IAAH, the protonated form of IAA; IAA^-, the IAA anion.

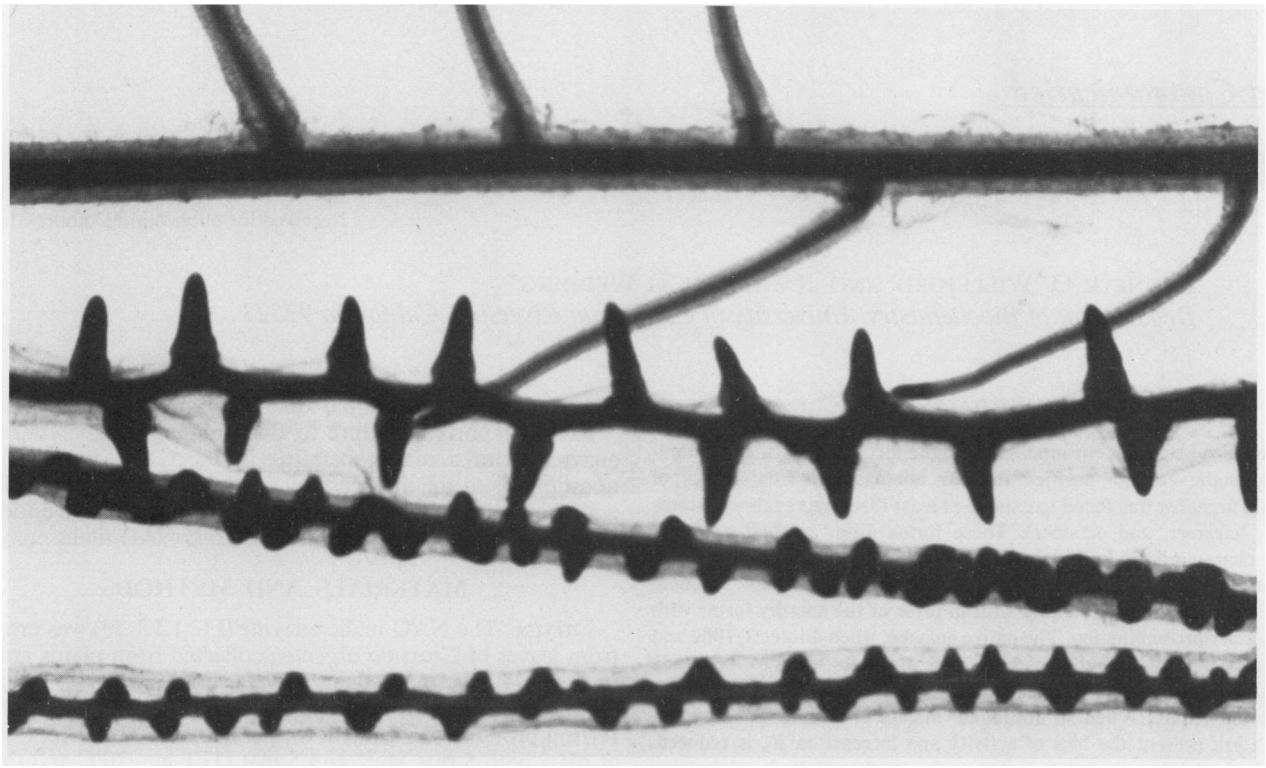

FIG. 1. One cm central regions of 2.5 cm segments cut from radish seedling roots, shown after 4 d of culture. The segment at the top was cultured in medium lacking IAA; the others were cultured in media containing 30 mmol m^{-3} IAA. The segment second from the top was cultured in medium buffered at pH 6. The segment below it was likewise cultured in medium buffered at pH 6; however, the first 2 h of culture was carried out in medium containing 5% (v/v) DMSO, then the segment was transferred to medium lacking DMSO for the remainder of the 4 d culture period. The segment on the bottom was cultured in medium buffered at pH 4.5. See the text for treatment mean LRP frequencies. (Treatment mean LRP developmental stage [1] typically declines as treatment mean LRP frequency increases.)

barrier occurring at the endodermis, and (b) the plasmalemma of the symplast external to an endodermal apoplastic barrier.

When using the radish seedling root system to perform quantitative studies on the effect of exogenous IAA (or other barrier-limited chemicals) on LR formation, the presence of the permeation barrier can complicate interpretation. If the effect of a treatment were to alter the permeation barrier, rather than or in addition to altering the response to IAA (or other chemical), misinterpretations might be made if the presence of the barrier were not recognized.

We have found that the medium pH can be changed substantially by sterilization and by the actions of the roots, in the absence of pH buffers. For quantitative studies involving IAA, adequate buffers should be employed so that the IAA form can be regulated. Buffers have not been routinely used in previous quantitative studies on auxin-induced LR formation.

The roots of the monocot *Pontederia cordata* did not form additional laterals in response to exogenous auxin (2). In other work from our laboratory (5) it was likewise found that the roots of intact seedlings of several species (both monocots and dicots) failed to respond to 40 mmol m^{-3} IAA solutions. Failure to respond to exogenous IAA could be due to (a) failure of IAA permeation, (b) insensitivity to IAA on the part of the pericycle, or (c) some other effect such as auxin degradation. DMSO treatment, and variation of medium pH, should be helpful in any studies to determine whether failure of permeation was a factor in these cases.

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